The Magnitude of Androgen Receptor Positivity in Breast Cancer is Critical for Reliable Prediction of Disease Outcome

Carmela Ricciardelli[1,2*], Tina Bianco-Miotto[1,2,3*], Shalini Jindal[1*], Lisa M. Butler[1], Samuel Leung[4], Catriona M. McNeil[5], Sandra A. O'Toole[5], Esmaeil Ebrahamie[1], Ewan K. A. Millar[5], Andrew J. Sakko[1], Alexandra I. Ruiz[1], Sarah L. Vowler[6], David G. Huntsman[7], Stephen N. Birrell[1], Robert L. Sutherland[5], Carlo Palmieri[8,9], Theresa E. Hickey[1] and Wayne D. Tilley[1]

* These authors contributed equally to this work

[1]Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia; [2]Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia; [3]School of Agriculture, Food and Wine, University of Adelaide, Adelaide, South Australia, Australia; [4]Genetic Pathology Evaluation Centre, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [5]Cancer Research Program, Garvan Institute of Medical Research, Sydney, New South Wales, Australia; [6]Cancer Research UK Cambridge Institute, University of Cambridge, Robinson Way, Cambridge, UK; [7]Department of Pathology and Laboratory Medicine, University of British Columbia, BC Cancer Agency, Vancouver, BC, Canada, [8]Institute of Translational Medicine, University of Liverpool, Liverpool, UK; [9]Academic Department of Medical Oncology, Clatterbridge Cancer Centre NHS Foundation Trust, Wirral, UK.

Correspondence to: Prof Wayne Tilley or Dr Theresa Hickey, Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, University of Adelaide, Adelaide, South Australia 5005, Australia. Tel: +61 8 8313 7861*; E-mail: wayne.tilley@adelaide.edu.au*

Running Title: AR robustly predicts outcome in breast cancer

Key words: androgen receptor, estrogen receptor, breast cancer prognosis

Total number of figures and tables: 3 Figures, 3 Tables, 3 Supplementary Figures, 7 Supplementary Tables

Statement of Translational Relevance

The estrogen receptor alpha (ERα) is routinely assessed in breast cancer to inform clinical management. More recently, the androgen receptor (AR) has been shown to be of potential prognostic value, but the appropriate cut-point for robust prognostication has yet to be established. In this study, an optimal cut-point for AR was determined using ROC analysis with a test cohort and its prognostic capacity validated in an independent cohort. Prognostic capacity was robust with a high (78% positivity) cut-point but not with lower (1% - 10%) positivity cut-points commonly used in previous studies. The 78% cut-point was valid for unselected cases and selected ERα positive cases. Among the latter, an AR:ERα positivity ratio indicative of comparable receptor expression or AR predominance was associated with the best survival outcome. Determination of the AR status in addition to ERα in breast tumors provides important prognostic information providing an optimal cut-point is utilized.

Abstract

Purpose: Consensus is lacking regarding the androgen receptor (AR) as a prognostic marker in breast cancer. The objectives of this study were to comprehensively review the literature on AR prognostication and determine optimal criteria for AR as an independent predictor of breast cancer survival.

Experimental Design: AR positivity was assessed by immunostaining in two clinically-validated primary breast cancer cohorts (training cohort n=219; validation cohort n=418; 77% and 79% estrogen receptor alpha (ERα) positive, respectively). The optimal AR cut-point was determined by ROC analysis in the training cohort and applied to both cohorts.

Results: AR was an independent prognostic marker of breast cancer outcome in 22/46 (48%) of previous studies that performed multivariate analyses. Most studies used cut-points of 1% or 10% nuclear positivity. Herein, neither 1 nor 10% cut-points were robustly prognostic. ROC analysis revealed a higher AR cut-point (78% positivity) provided optimal sensitivity and specificity to predict breast cancer survival in the training (HR 0.41, P=0.015) and validation (HR 0.50, P=0.014) cohorts. Ten-fold cross validation confirmed the robustness of this AR cut-point. Patients with ERα positive tumors and AR positivity $\geq$78% had the best survival in both cohorts (P<0.0001). Among the combined ERα positive cases, those with comparable or higher levels of AR (AR:ERα positivity ratio >0.87) had the best outcomes (P<0.0001).

Conclusions: This study defines an optimal AR cut-point to reliably predict breast cancer survival. Testing this cut-point in prospective cohorts is warranted for implementation of AR as a prognostic factor in the clinical management of breast cancer.

Introduction

Androgen receptor (AR) expression is highly prevalent in primary breast cancers, ranging from 53-99% depending on the characteristics of the cohort analyzed, the assay methodology and the criteria for positivity (1-7). Up to 90% of estrogen receptor alpha (ERα) positive tumors and approximately 20% of ERα negative tumors are also AR positive (1-7). Given the high frequency of AR expression in breast cancer and the availability of old and new generation agents to modulate its activity, there has been a resurgence of interest in targeting the AR signaling pathway to treat women with this disease. However, much controversy exists over how best to target AR in breast cancer as it appears to have pleiotropic roles dependent on disease subtype and stage of progression (reviewed in (8) and (9)). Strong clinical and preclinical evidence supports AR as being growth inhibitory in hormone sensitive, ERα positive breast malignancies, a role that may be sustained from normal breast tissue as AR activity inhibits breast development in men and women (8, 10). Accordingly, non-aromatizable AR agonists such as fluoxymesterone have historically demonstrated an efficacy comparable to that of the selective ERα modulator, tamoxifen, in advanced breast cancer (11, 12). However, use of androgenic agents was discontinued due to virilizing side effects in some women. Development of new selective AR modulators (SARMs) with AR agonist activity in breast tissues may circumvent that problem and one, Enobosarm (GTx024), has shown promise in a phase II trial of women with advanced, hormone sensitive disease (13). This approach is supported by a recent preclinical study in which induction of AR agonist activity using a different SARM inhibited the growth of endocrine-sensitive as well as endocrine-resistant patient-derived breast cancer xenografts (14). However, some pre-clinical studies have suggested that AR antagonism is also a therapeutic option for women with ERα positive disease (15-17), leading to breast cancer trials with new generation AR antagonists such as enzalutamide (e.g. ClinicalTrials.gov Identifier: NCT02953860) currently used to treat prostate cancer. Antagonism of AR activity may have a clinical niche in the treatment of ERα negative, AR positive disease, particularly the triple negative breast cancer (TNBC) subtype, in which AR is

purported to have oncogenic activity (18-24). However, at μM concentrations AR antagonists can have off-target effects in breast cancer cells (25), suggesting that their therapeutic efficacy may not be mediated by inhibition of AR.

Numerous studies have investigated whether AR is a biomarker for predicting survival of breast cancer, but like the potential utility and means of targeting AR, its prognostic value remains controversial. Disparate results among studies may be attributed to heterogeneity of breast cancer cohorts and differences in methodology, including the use of different cut-points for AR positivity. Although several meta-analyses commonly conclude that AR is associated with better outcomes in ERα positive disease (26-29), these analyses used population-level rather than individual patient-level data. Moreover, most studies included in the meta-analyses of AR as a prognostic biomarker used cut-points that are typically those used for ERα and progesterone receptor (PR) as a predictive biomarker (30, 31). To date, no standardized cut-point for AR prognostication has been statistically defined, which most likely has contributed to conflicting results in the literature regarding AR as a prognostic biomarker in breast cancer.

In the current study, we comprehensively reviewed the literature on AR prognostication for breast cancer survival to highlight methodological heterogeneity in previous studies, and utilized two clinically validated breast cancer cohorts from Australia (32) and Canada (33) with long-term follow-up to empirically define optimal criteria for AR to be a robust independent predictor of breast cancer specific survival. Since there has been interest in assessing the clinical relevance of different AR to ERα expression ratios in breast cancer (8, 15, 34, 35), we also evaluated this parameter.

Methods

Study selection for comprehensive review on AR and breast cancer specific outcome

Primary publications that investigated the relationship between AR expression and breast cancer outcome were identified by searching PubMed with the terms ‘breast cancer’ and ‘androgen

receptor' up to July 2017. Only studies that examined the relationship between AR level and patient outcome were included; there was no restriction based on methodology used to assess AR protein levels. Exclusion criteria for the PubMed search included the following 1) non-English articles, 2) review articles, 3) no information provided for at least one of the following: disease free survival (DFS), relapse-free survival (RFS) or breast cancer specific overall survival (OS) and 4) duplicate publications/cohorts. Our comprehensive review identified a total of 53 articles (Table 1). Articles were divided into 3 cohort subtypes: (1) unselected (i.e. all cases); (2) ERα positive and (3) ERα negative cohorts, including TNBC.

Patient Cohorts

Prior approval for this study was obtained from the Human Research Ethics Committee of the University of Adelaide (H-051-2006). Two independent breast cancer cohorts (32, 33) were studied, both represented on tissue microarrays (TMA) consisting of replicate sample cores. For detailed pathological and clinical characteristics see Supplementary Table 1 and for details of immunostaining methods, statistical analyses and cut-point determination see Supplementary Methods.

Training cohort: 219 patients with invasive ductal breast carcinoma diagnosed between 1992 and 2002 from St Vincent's Hospital, Sydney, Australia (32). Prior approval for this TMA construction was obtained from the Human Research Ethics Committee of St Vincent's Hospital, Sydney. Median follow up was 90 months.

Validation cohort: 418 patients with invasive breast cancer diagnosed at Vancouver General Hospital, between 1974 and 1995 (33). TMAs were constructed at the Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, Canada with ethical approval from the Institutional Ethical Review Board. Median follow up was 143 months.

Immunostaining and AR antisera

Sections of paraffin-embedded breast cancer tissue (4 μm) from the two TMA cohorts were immunostained with two different AR antibodies, U407 (epitope: amino acids 200-220) (36) for the

training cohort) and AR-N20 (SC-816, epitope: amino acids 1–20; Santa Cruz; Dallas, TX) for the validation cohort. The specificity of both of these AR antibodies has been confirmed previously by western blot and peptide competition experiments (36, 37). Concordance of immunostaining with the two AR antibodies is shown in Supplementary Figure 1. Details of the AR immunohistochemistry methodology and scoring are contained in Supplementary Methods. Ki67 (SP6, Neomarkers, USA; 1:200 dilution) was scored as a visual estimate of continuous percent positivity by a board certified clinical pathologist for both cohorts (38, 39).

ROC Analysis

ROC analysis was used to dichotomize AR and Ki67 positivity for all cases in the individual training and validation cohorts. Additional ROC analyses were performed for all cases and the ERα positive cases in the combined training and validation cohort. There were insufficient cases in the combined cohort to perform ROC analyses for HER2+ or ERα negative sub-groups. The optimal cut-points for predicting breast cancer death were determined using the Youden index (J), which was calculated using the formula J = max [sensitivity + specificity-1] (40). In addition to ROC analysis, recursive partitioning was applied to the training cohort to select the most appropriate AR cut-point (41). For additional details of the statistical analyses see Supplementary Methods. Statistical power of the ROC-derived AR cut-point for each of the cohorts was determined using Minitab 17 (www.minitab.com, USA).

Ten-fold Cross Validation

Ten-fold cross validation was performed to provide evidence of the accuracy of the classifier model to correctly predict the AR classes generated by ROC analysis (42-44). To this end, we employed supervised learning to build a range of classifiers including Decision Stump, Decision Tree, Decision Tree with information gain and Naïve Bayse from cohort features such as tumor size and overall survival. Ten-fold cross-validation randomly divides the dataset into 10 sub-samples and

uses 9 sub-samples as training data and one sub-sample as test data. This process is repeated 10-times. Cross-validation is a reliable statistic in supervised learning and class prediction as it avoids overlapping test sets. The above-mentioned analyses were performed using RapidMiner 6.0 software (RapidMiner, Boston, USA).

Results

A comprehensive analysis of studies investigating AR as a biomarker of breast cancer survival

A comprehensive review of the literature on AR as a prognostic factor in breast cancer was undertaken. A total of 53 studies that assessed AR status and disease outcome in breast cancer using either radio-ligand binding assays (n=7), immunostaining (n=45), or reverse phase protein microarray (n=1) were identified in a PubMed search with the criteria outlined in Methods (Table 1). Of these, 7 did not perform multivariate analyses. AR was predictive of breast cancer outcome in 22/46 (48%) of all previous studies that performed multivariate analyses. Nearly all previous studies used cut-points of 1% or 10% nuclear AR positivity. Among the 22 studies involving unselected breast cancer cohorts, most found that AR is prognostic for OS by univariate analysis, but only 10/22 (45%) identified AR as an independent prognostic marker of OS by multivariate analyses (Table 1). Among the 8 studies involving cohorts selected for ERα positive disease, 5/8 (62%) identified AR as an independent predictor of outcome. Findings in cohorts selected for ERα negative disease were most conflicting, with a significant association between AR expression and OS reported in only 7/18 (39%) of studies. Of note, 5 of these 7 studies (45-49) reported that AR expression conferred a survival advantage in tumors that lacked ERα expression, but 2 (50, 51) reported a survival disadvantage (Table 1). Collectively, the studies published to date highlight the lack of consensus on whether AR is an independent prognostic factor for breast cancer survival in unselected or selected cohorts. Importantly, none of the published studies performed ROC analysis or used an alternative statistical approach to define the optimal cut-point for AR prognostication in breast cancer. The majority of studies employed an arbitrary cut-point, the most common being 1% or 10% AR positivity. While these cut-points, commonly used for ERα and PR as a predictive

biomarker (30, 31), may be useful to determine AR status, they are not necessarily optimal for use in prognostication.

Commonly used cut-points of AR positivity do not reliably predict breast cancer survival

The majority (>75%) of breast cancers in the training and validation cohorts analyzed in this study were classified as having AR positive nuclear immunostaining irrespective of whether a 1% or 10% criterion was used (Supplementary Table 1). ROC analysis and the generated area under the curve (AUC) for all cases was used to assess how well AR positivity could distinguish between patients that died from breast cancer and those that survived at least 10 years. AR positivity had prognostic capacity in both the training (AUC=0.678, 95% CI; 0.587-0.770, P<0.0001, Figure 1A) and validation (AUC=0.588, 95% CI; 0.525-0.651, P=0.008, Figure 2A) cohorts. Based on the ROC analysis, a cut-point of 1% AR positivity, used in 14/36 of previous studies (see Table 1), resulted in high sensitivity for predicting breast cancer OS in the training (90.0%) and validation (93.8%) cohorts, but a low specificity in both instances (training: 27.5%; validation: 7.8%). Similarly, the commonly used cut-point of 10% AR positivity (13/36 of studies in Table 1) resulted in high sensitivity (training: 84.7%; validation: 85.8%) but low specificity (training: 35.0%; validation: 18.4%) for predicting OS. Kaplan-Meier and Cox regression analyses indicated that nuclear AR positivity arbitrarily assigned as either $\geq$1% or $\geq$10% positivity was significantly associated with OS in the training cohort but not in the validation cohort (Supplementary Figure 2, Table 2, Table 3).

Defining a cut-point for AR positivity that confers robust prognostication

As neither a 1% nor a 10% cut-point for AR positivity was prognostic for breast cancer OS in both the training and validation cohorts, the Youden index method, which gives equal weight to sensitivity and specificity, was applied to the ROC analysis to identify an optimal AR cut-point for

all cases in the training cohort. The highest Youden index was obtained using 77.5% AR positivity, which resulted in a sensitivity of 57.1% and a specificity of 75.0% for predicting breast cancer death. This cut-point resulted in a positive predictive value of 90.7%. An alternative method, recursive partitioning, identified 77% as the optimal cut-point for the training cohort to predict OS. The ROC determined cut-point of 77.5% (rounded to 78%) was significantly associated with ERα status and breast cancer subtype in both cohorts (Supplementary Tables 2 and 3, Chi Squared test). The frequency distributions for AR percent positivity and representative images of tumors classified as either high (≥78% positivity) or low (<78%) AR positivity are shown for both the training and validation cohorts in Figure 1B-C and Figure 2B-C, respectively. Most tumors (69.4%) with AR positivity <78% were also ERα negative (i.e. <1% ERα positivity) in the training cohort; 73.6% were ERα negative in the validation cohort. A high proportion of TNBC had low AR positivity in both the training (79%) and validation (69%) cohorts (Supplementary Tables 2 and 3). Ten-fold cross validation was performed using all cases to provide evidence of the accuracy of the classifier model to correctly predict the two AR classes (i.e. <78% and ≥78% positivity) generated by ROC analysis. A high accuracy of 10-fold cross validation (>80%) for most of the classifiers demonstrated the robustness of AR positivity equal to 78% as an optimal cut-point based on cohort features in both the individual and combined training and validation cohorts (Supplementary Table 4). For example, for the combined training and validation cohorts, the accuracy of Decision Tree with information gain criterion in prediction of AR classes <78% and ≥78% was 87.27% and 90.98%, respectively (Supplementary Table 4).

Histological grade III and progesterone receptor (PR) negative tumors were significantly associated with low AR positivity in the training cohort (Supplementary Table 2). The lack of an association between AR and PR in the validation cohort (Supplementary Table 3) is possibly the result of approximately one third of the cases having unknown PR values (150/418; 35.9%), whereas PR was measured in almost all the cases (214/219; 97.7%) in the training cohort (Supplementary Table 1).

Kaplan-Meier survival analyses for all cases demonstrated that an AR positivity ≥78% was significantly associated with OS in both the training (Figure 1D, *P*<0.0001) and the validation (Figure 2D, *P*=0.001) cohorts. Patients with tumor AR positivity ≥78% had approximately a 3-fold reduced risk of cancer-related death in the training cohort (HR=0.32, Cox regression analysis, *P*=0.001, Table 2) and a 2-fold reduced risk in the validation cohort (HR=0.51, Cox regression analysis, *P*=0.001, Table 3). AR immunostaining with a cut-point of 78% was an independent predictor of OS after adjusting for all other variables significant by univariate analysis in both cohorts (Tables 2 and 3) but was not an independent predictor of RFS (Table 2). Neither the 1% nor 10% AR cut-points were significant by univariate analysis in the validation cohort (Table 3).

AR prognosis with adjuvant therapy

Treatment information was available for the training cohort but unavailable for the validation cohort. In the training cohort, AR with a 78% cut-point predicted OS (P=0.002, log rank statistic=9.63) in patients who received adjuvant chemotherapy (n=82; Supplementary Figure 3A), but not in those who did not receive adjuvant chemotherapy (n=128; P=0.067; Supplementary Figure 3B). AR was a significant predictor of OS in all patients irrespective of whether they did (n=110, P=0.014, log rank statistic=6.10, Supplementary Figure 3C) or did not (n=100, P=0.010, log rank statistic=6.69, Supplementary Figure 3D) receive adjuvant endocrine therapy. Similarly, AR predicted outcome in the ERα positive subgroup irrespective of whether patients received adjuvant endocrine therapy (n=89, P=0.01, log rank statistic=6.59) or not (n=67, P=0.017, log rank statistic=5.74).

ERα positive breast cancers with high AR have the best survival outcome

In both the training and validation cohorts, patients with ERα positive tumors (ERα ≥1%) that contained high levels of AR (i.e. ERα+, AR ≥78%) had significantly increased OS in comparison to those in the other 3 possible ERα and AR classification groups (i.e. ERα positive and AR <78%,

ERα negative and AR ≥78%, ERα negative and AR <78%; Figure 1E and 2E). Patients with ERα negative disease (ERα <1%) had an increased risk of death regardless of AR status, compared to those with ERα positive AR ≥78% tumors (training cohort: 11.5 and 10.3-fold risk, *P*<0.0001, Figure 1F; validation cohort: 1.9 and 2.9-fold risk, *P*=0.006 and *P*<0.0001, Figure 2F). The training and validation cohorts were combined for greater statistical power to assess the ability of AR to predict OS as a continuous variable or with an AR cut-point of <78% or ≥78% positivity. AR levels predicted OS in ERα positive tumors but not ERα negative tumors (Supplementary Table 5). When assessed by subtype, AR was an independent predictor of OS in Luminal A but not Luminal B cancers (Supplementary Table 5). When ROC analysis was applied to all cases or just the ERα positive cases in the combined training and validation cohorts, the optimal AR cut-point was 77.95%.

The AR to ERα ratio is a determinant of OS in ERα positive disease

To investigate whether the relative levels of AR and ERα influenced OS, the training and validation cohorts were combined for greater statistical power and unbiased tertiles were calculated for the AR to ERα positivity ratio (<0.87, 0.87-1.05 and >1.05). Kaplan-Meier and Cox regression analyses demonstrated that patients with tumors containing comparable levels of AR and ERα (i.e. an AR to ERα positivity ratio approximating 1; range 0.87-1.05), or a predominance of AR (i.e. an AR to ERα positivity ratio >1.05) had the highest 10-year breast cancer survival outcomes (83.3% and 80.5%, respectively; *P*<0.0001; Figure 3). In contrast, patients with a predominance of ERα (i.e. an AR to ERα positivity ratio <0.87) had a poorer survival outcome compared to patients with similar AR and ERα immunostaining levels (10-year breast cancer survival 71.6%; Figure 3; *P*<0.0001). Patients with tumors that were either AR or ERα negative or lacked both receptors had a lower 10-year breast cancer specific survival rate of 53-66% (Figure 3; 3-fold increased risk of death, P<0.004). ROC analysis showed that the optimal cut-point for the AR to ERα positivity ratio was 0.82. However, in multivariate analyses, neither the AR to ERα ratio tertile groups nor an AR to

ERα ratio cut-point of 0.82 were independent predictors of OS in the combined training and validation cohorts (Supplementary Table 6A and 6B). Consistent with this, the AR to ERα ratio did not differentiate between luminal A and luminal B ERα positive breast cancer subgroups (Supplementary Table 7).

Discussion

The current study demonstrates that clinical assessment of AR in addition to ERα may permit a more precise prediction of breast cancer outcome in women, particularly those with hormone sensitive disease, providing an optimal cut-point for AR is utilized. As highlighted by our comprehensive review of the literature, many published studies have investigated AR as a prognostic factor in breast cancer, but the findings have been inconsistent resulting in doubt about its clinical utility for prognostication. A critical common limitation of previous studies has been the failure to define an optimal AR cut-point for prognosis. Rather, arbitrary cut-points of 1% and 10% positivity, used to determine AR status, were typically applied to dichotomize data for prognostic testing. Herein, we provide the first robust, statistically derived cut-point for future testing of AR as a prognostic factor in prospective cohorts.

ROC analysis using two independent, well characterized breast cancer cohorts with 10-year follow-up demonstrated that a cut-point of 78% AR positivity, which approximated the median value, achieved the best combination of specificity and sensitivity for prediction of breast cancer survival. This cut-point value was reinforced using recursive partitioning of the data. Ten-fold cross validation, a statistic used in supervised learning and class prediction, confirmed the robustness of the AR cut-point defined by ROC analysis. Our finding that the optimal AR cut-point approximated the median percentage positivity determined by immunostaining is consistent with 2 of 3 previous biochemical (radio-ligand binding) studies of unselected breast cancer cohorts that arbitrarily used the median AR protein level to dichotomize the breast cancer survival data (52-54). More recently,

Tokunaga et al (7) employed an arbitrary cut-point of 75% AR positivity, which approximated the median immunostaining, to demonstrate that AR is an independent predictor of outcome in an unselected breast cancer cohort. Hence, our statistically determined AR cut-point is not without precedence in the literature concerning AR and breast cancer prognostication when all cases are considered.

AR was prognostic in this study for all breast cancer cases in both cohorts, which likely reflects its negative correlation with tumor size and proliferative capacity, regardless of ERα status as shown in this study and previously reported (3, 5, 55). A recent meta-analysis in which AR mRNA expression was correlated with various gene signatures in large combined breast cancer cohorts strongly supports this concept (26). Functionally, AR and ERα are hormone-activated nuclear transcription factors that regulate gene expression and are commonly co-expressed in normal and malignant breast epithelial cells (37, 56), indicating the potential for direct cross-talk between these two sex hormone receptor signaling pathways. Indeed, antagonism between AR and ERα signaling is thought to underpin sex-specific breast development (9). In breast cancer, this interaction and its functional consequence is likely to be perturbed by altered receptor levels and a pathological hormone milieu (8, 9, 34).

Our data showing that a high AR cut-point is required to robustly predict survival from ERα positive disease is consistent with the concept that a minimum threshold of AR activity is required to restrain growth of ERα positive breast cancers (34, 37). Indeed, this may explain why AR positivity tends to be higher than ERα positivity in normal and most ERα positive malignant breast tissues, even though the expression of both receptors is increased in the malignant compared to the normal state (8). We have previously shown that exogenous AR dose-dependently inhibited ERα transcriptional activity from an AR to ERα molar ratio of 1:1 to 4:1 (37). This finding is in accordance with another study showing that an AR to ERα ratio approximating 1 was associated with a survival advantage in a tamoxifen-treated cohort of ERα positive patients (15). However, the

same study disagreed with our finding that a predominance of AR over ERα conferred a survival advantage, reporting that an AR to ERα ratio > 2 was associated with treatment failure on tamoxifen (15). In that study, AR positive tumors were defined as AR >0% positivity, which has only been used in 1 other immunohistochemical study (see Table 1), and AR alone did not independently predict outcome. Moreover, the AR=0% cases, which are well established as being associated with a poor prognosis, were included in the AR to ERα ratio <2 group. In our study, in the analysis showing that patients with tumors characterized by a predominance of ERα over AR had a reduced chance of survival, only tumors that were positive for both AR and ERα were included in the analysis. In this scenario, AR may have lost its capacity to antagonize ERα signaling, either due to insufficient expression or activation. Additionally, a recent preclinical study showed that estrogen potentially can activate AR in a non-classical manner to facilitate ERα genomic activity in breast cancer cells (17). Hence, studies examining the prognostic value of AR in ERα positive breast cancers that included tumors with very low levels of AR expression (e.g. cut-points of >0%, >1% or >10%) may have produced disparate results because low AR expression or insufficient activation cannot effectively oppose ERα activity or conditions are such that AR is hijacked into facilitating ERα activity. This presents a therapeutic conundrum as the latter scenario indicates an AR antagonist strategy whereas the former suggests an AR agonist strategy. While both options are supported by recent studies that have employed patient-derived xenograft models (14, 17), the outcome of current clinical trials will provide definitive evidence regarding this controversy.

AR status did not improve the stratification of ERα negative breast cancers in terms of patient outcome, likely due to insufficient power. In a larger study, Hu et al (3) showed that postmenopausal women with ERα negative breast cancers had poor survival irrespective of AR status. Since ERα negative breast cancers are less common than ERα positive cancers and represent a very heterogeneous mix of molecular disease entities, it has been difficult to definitively identify robust prognostic factors. However, meta-analyses of studies examining AR as a prognostic factor

in breast cancer have found that AR is associated with a better outcome in ERα negative disease in general or of its various sub-groupings (27-29). Despite the reported association of AR with a better outcome in this disease context, there has been much enthusiasm for therapeutic targeting of AR, particularly in TNBC, as there are currently no targeted therapies for the treatment of this aggressive disease subtype. The mainstream clinical approach has been AR antagonism based on *in vitro* and *in vivo* pre-clinical studies suggesting that AR has oncogenic activity in some ERα negative breast cancer models (18-24, 57), and two clinical trials of women with advanced TNBC have reported efficacy with this approach (58, 59). However, *in vitro* studies have demonstrated dichotomous proliferative effects of androgens in different AR positive ERα negative breast cancer cell lines (8). For example, while the MDA-MB-453 breast cancer cell line is stimulated by androgen *in vitro* (15, 20-22), other AR positive ERα negative breast cancer cell lines (MFM-223 and CAL-148) are inhibited (25, 60). Androgenic stimulation of tumor growth has also been demonstrated *in vivo* using MDA-MB-453 xenografts (15, 22), but this has not been convincingly demonstrated with other ERα negative models. Rather, these studies have inferred a growth stimulatory effect of AR signaling *in vivo* by showing growth inhibition using an AR antagonist (bicalutamide or enzalutamide) alone (18, 23, 24). The interpretation of these studies is confounded by the potential for off-target effects of AR antagonists at μM doses in ERα negative breast cancer cells (25).

Although the two cohorts in this study have the advantage of long-term clinical follow-up, treatment practices have evolved since the cohorts were assembled. Nevertheless, adjuvant endocrine therapy with tamoxifen or new generation ERα-target therapies remains a critical component of standard-of-care for women with ERα positive disease. A sub-analysis of the training cohort, where treatment information was available, demonstrated that AR, using a 78% cut-point, predicted OS in patients who received tamoxifen as adjuvant endocrine therapy. Considering our findings that AR is a robust independent prognostic factor for breast cancer survival in both unselected and selected ERα positive cases providing an appropriate cut-point was utilized, we

propose that studies to determine the optimal AR cut-point for selection of patients who are likely to respond to hormonal interventions targeting either the AR or ERα are warranted. This is particularly important given the recent interest in targeting AR in breast cancer, with clinical trials initiated to investigate either the stimulation or inhibition of this signaling pathway (ClinicalTrials.gov identifiers NCT00468715, NCT01597193, NCT00755885, NCT01889238, NCT01616758, NCT02463032 and NCT02007512). At present, there is no consensus regarding how best to select breast cancer patients who most likely will benefit from an AR targeted therapeutic intervention, highlighting the need for well-designed and validated prospective studies of AR as a predictive marker as well as a prognostic marker.

In summary, by eliminating the use of arbitrary criteria for AR positivity to predict breast cancer survival, assessment of AR status could become an important clinical tool in the management of this disease. Since pre-clinical studies suggest a role for AR in resistance to tamoxifen (15, 61) and aromatase inhibitors (62, 63), it will be important to test the prognostic power of the AR in the context of the cut-points defined herein using tissues collected from large contemporary, prospective breast cancer cohorts.

Acknowledgments

This paper is dedicated to Robert L. Sutherland, a long-time colleague and friend, who died on 10 October 2012 of pancreatic cancer. The authors would like to thank Sarah Dawson, Cambridge Institute, Cambridge University, Cambridge UK and Shalem Leemaqz, University of Adelaide, for statistical advice and Marie Pickering for technical support.

Financial Disclosure: This work was supported by grants from the National Health and Medical Research Council of Australia (NHMRC) (ID: 1008349 WDT; 1084416 WDT and TEH; Program Grant 535903 EKAM, SAO'T, RLS; Fellowship 427601 RLS), Cancer Australia/National Breast

Cancer Foundation (ID 1043497; WDT, TEH), the Susan G. Komen for the Cure (ID: BCTR0601118; WDT; SNB; TBM) and a National Breast Cancer Foundation Novel Concept Award (NC-08-12; TEH). WDT is also supported by a grant from the National Breast Cancer Foundation (PS-15-041). CR was supported by the Hilda Farmer Research Fellowship (University of Adelaide Faculty of Health Sciences) and is currently supported by the Lin Huddleston Ovarian Cancer Fellowship (Cancer Council South Australia and Adelaide Medical School, University of Adelaide). TBM was supported by the NHMRC Peter Doherty Fellowship (#349556). TEH held a Fellowship Award from the US Department of Defense Breast Cancer Research Program (BCRP; #W81XWH-11-1-0592) and currently is supported by a Florey Career Development Fellowship from the Royal Adelaide Hospital Research Foundation. LMB is an Australian Research Council Future Fellow. Additional support was provided by the Cancer Institute of New South Wales (Translational Program Grant 10/TPG/1-04 RLS, EKAM, SAO'T; Sydney Catalyst Translational Research Centre 11/TRC/1-02 RLS; Fellowship 10/CRF/1-07 SAO'T), the Australia and New Zealand Breast Cancer Trials Group (RLS, SAO'T), the Australian Cancer Research Foundation (RLS), the Sydney Breast Cancer Foundation (SAO'T), the RT Hall Trust (RLS) and the Petre Foundation (RLS). The Genetic Pathology Evaluation Centre is supported by an unrestricted educational grant from Sanofi-Aventis. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript, for which the authors of this study had full responsibility.

Competing Interests: The authors have no conflicts to disclose.

Tables

Table 1. Summary of studies investigating Androgen Receptor as a prognostic factor in breast cancer.

Cohorts not selected on ERα status								
Ref	**Year**	**N**	**Sample**	**Ab**	**Cut-point**	**Univariate**	**Multivariate**	**HR (95%CI)**
(64)	1979	292	RLB	-	≥10 fmol/mg	NS	ND	
(65)	1984	1181	RLB	-	≥5 fmol/mg	OS (P<0.001)	ND	
(66)	1986	796	RLB	-	≥5 fmol/mg	OS (P<0.05)	ND	
(67)	1990	61	RLB	-	≥10 fmol/mg	OS at 36 months (P=0.043)	ND	
(54)	**1992**	**224**	**RLB**	**-**	**≥50.5 fmol/mg (median)**	**Yes**	**MFS (P=0.001)**	**NA**
(52)	1996	269	RLB	-	>43 fmol/mg (median)	NS	ND	
(68)	1996	153	Frozen - WS	ARF39.3	≥10%	DFS (P=0.043)	NS	
(69)	2006	232[a]	TMA	AR441	>10%	DFS (P=0.028)	NS	
(70)	2007	115	FFPE – WS	AR441	>10%	OS (P=0.03)	NS	
(71)	2007	1087	TMA	AR441	Allred score >3	NS	NS	
(72)	2008	488	FFPE – WS	AR441	NS	RFS (P=0.023)	NS	
(73)	**2008**	**111**	**TMA**	**AR441**	**>0**	**OS (P = 0.01)**	**OS (P = 0.03)**	**0.46 (0.23–0.93)**
(74)	2008	138	FFPE – WS	AR441	≥15%	OS (P=0.01)	NS	
(74)	**2008**	**138**	**RLB**	**-**	**≥30 fmol/mg**	**RFS (P=0.007) OS (P=0.007)**	**RFS (P<0.0001) OS (P=0.003)**	**0.36 (0.22-0.59) 0.4 (0.21-0.71)**
(53)	**2009**	**347**	**RPA**	**NR**	**≥-0.085 (median)**	**RFS (P=0.002) OS (P=0.004)**	**RFS (P=0.002) OS (P=0.013)**	**0.53 (0.36-0.80) 0.57 (0.36-0.89)**
(75)	2011	673	TMA	ARF39.4.1	Remmele score ≥3	RFS (P=0.033) OS (P=0.023)	NS	
(76)	**2011**	**335**	**FFPE – WS**	**AR441**	**Allred score NR**	**OS (P<0.001)**	**OS (P<0.001)**	**0.31 (0.19-0.50)**
(3)	2011	1467	TMA	AR441	≥1%	Yes	NS	
(77)	2012	73[b]	TMA	AR441	≥1%	OS (P=0.004)	ND	
(2)	2012	403	FFPE – WS	AR27	≥10%	DFS (P = 0.017) OS (P = 0.034)	NS	
(7)	**2013**	**250**	**NR**	**AR441**	**≥75%**	**DFS (P=0.0005)**	**DFS (P=0.005)**	**0.46 (0.26-0.79)**
(28)	**2013**	**109**	**TMA**	**AR441**	**>1%**	**DFS (P=0.026) OS (P=0.022)**	**DFS (P=0.031) OS (P=0.031)**	**0.24 (0.07-0.88) 0.19 (0.04-0.86)**
(78)	**2013**	**379**	**NR**	**AR-318**	**Histoscore >10**	**RFS (P<0.0001) OS (P<0.0001)**	**RFS (P<0.0001) OS (P=0.0059)**	**0.24 (0.12-0.50) 0.28 (0.10-0.70)**
(79)	2014	1039	TMA	AR441	≥1%	OS (P=0.002)	ND	
(80)	2014	807	TMA	AR441	≥1%	OS (P=0.001)	ND	
(81)	2014	82	FFPE – WS	AR441	Histoscore ≥3	OS (P=0.042)	ND	
(82)	**2015**	**1100**	**TMA**	**ARF39.4.1**	**Histoscore**	**Yes**	**OS (P=0.009)**	**0.65 (0.47-0.90)**
(35)	2015	1026	TMA	AR441	>10%	DFS (P=0.025)	NS	
(55)	**2016**	**1141**	**TMA**	**AR-N20**	**H-score >190**	**OS (P<0.001)**	**OS (P=0.033)**	**0.80 (0.64-0.98)**
ERα Negative Breast Cancers								
(83)	2003	69[c] ER-	FFPE – WS	ARF39.4.1	>5%	DFS (P=0.049)	NS	
(84)	2010	226[d] ER-PR-	FFPE – WS	AR27	≥10%	NS	NS	
(85)	2007	282[e] TNBC	TMA	ARF39.4.1	Modified H-score ≥1%	OS (P=0.04)	NS	
(47)	**2010**	**137[e] TNBC**	**FFPE – WS**	**NR**	**Score ≥2**	**OS at 5 years (P=0.018)**	**OS at 5 years (P=0.047)**	**NR**
(48)	**2011**	**127[f] TNBC**	**TMA**	**AR441**	**≥10%**	**OS (P=0.038)**	**OS (P=0.048)**	**NR**
(46)	**2012**	**287[f] TNBC**	**TMA**	**AR441**	**≥5%**	**DFS (P=0.008)**	**DFS (P=0.032)**	**0.47 (0.23-0.94)**
(86)	2012	83[g] TNBC	FFPE – WS	AR441	>1%	NS	NS	
(87)	2013	203[f] TNBC	FFPE – WS	AR441	H-score ≥10%	NS	ND	
(88)	2014	699[g] TNBC	TMA	AR27	≥1%	DFS (P=0.05)	ND	
(89)	2014	173[f] TNBC	TMA	ARF39.4.1	≥5%	OS (P=0.032)	NS	

(90)	2014	119[f] TNBC	TMA	AR441	≥10%	NS	ND	
(91)	2014	81[d] TNBC	FFPE – WS	ARF39.4.1	>10%	NS	ND	
(50)	**2014**	**492[g] TNBC**	**TMA**	**ER179 (2)**	**≥1%**	**OS (P=0.026)**	**OS (P=0.008)**	**2.16 (1.22-3.81)**
(92)	2014	52[f] TNBC	FFPE – WS	AR SP107	≥10%	DFS (P=0.11) OS (P=0.13)	ND	
(49)	**2015**	**45[e] TNBC**	**FFPE – WS**	**AR441**	**≥10%**	**OS (P=0.03)**	**OS (0.01)**	**0.15 (0.04-0.59)**
(93)	2016	120[g] ER-	FFPE – WS	ZM-0437	≥10%	DFS (P=0.21)	ND	
(51)	**2016**	**137[f] TNBC**	**TMA**	**AR441**	**>45%**	**DFS (P=0.017)**	**DFS (P=0.012)**	**2.42 (1.21-4.85)**
(45)	**2016**	**190[g] TNBC**	**FFPE – WS**	**AR441**	**≥1%**	**DFS (P=0.025)**	**DFS (P=0.039)**	**0.36 (0.14-0.95)**
ERα Positive Breast Cancers								
(37)	**2009**	**157[d]**	**TMA**	**AR-407**	**≥75%**	**RFS (P=0.011)** **OS (P=0.003)**	**RFS (P=0.003)** **OS (P=0.002)**	**0.33 (0.20-0.85)** **0.22 (0.08-0.58)**
(94)	**2010**	**859[g]**	**TMA**	**AR441**	**≥1%**	**RFS (P=0.001)** **OS (P<0.001)**	**RFS (P <0.0001)** **OS (P<0.0001)**	**0.46 (0.30-0.71)** **0.26 (0.14-0.48)**
(6)	**2011**	**672[d]**	**TMA**	**AR441**	**≥10%**	**DFS (P=0.005)** **OS (P=0.032)**	**DFS (P=0.049)** **NS**	**0.65 (0.43-1.00)**
(95)	**2013**	**543[g]**	**TMA**	**AR441**	**≥1%**	**OS (P<0.001)**	**OS (P=0.003)**	**0.26 (0.11-0.62)**
(96)	2014	798[g]	TMA	AR441	>1%	DFS (P=0.025)	NS	
(15)	2014	192[h]	FFPE – WS	AR441	>0%	NS	ND	
(97)	2015	96[d]	FFPE – WS	AR441	>10%	NS	ND	
(93)	**2016**	**120[g]**	**FFPE – WS**	**ZM-0437**	**≥10%**	**DFS (P=0.011)**	**DFS (P=0.01)**	**0.25 (0.09-0.72)**

Abbreviations: Ab, antibody; AR, androgen receptor; AR+, AR positive; AR-, AR negative; DFS, breast cancer specific relapse or breast cancer death; ER, estrogen receptor; FFPE, formalin fixed paraffin embedded; HR, hazard ratio; IHC, immunohistochemistry; MFS, metastasis free survival; NA, not available; ND, not determined; NR, not reported; NS, not significant; OS, overall survival or breast-cancer specific survival; PR, progesterone receptor; Ref, reference; RFS, relapse free survival; RLB, radio ligand binding assay; RPA, reverse phase protein microarray; TMA, tissue microarray; WS, whole section; [a]tumor samples from patients who developed metastatic disease; [b]grade III cancers; [c]ER cut-point >5%; [d]ER cut-point ≥10%; [e]ER cut-point >0%; [f]ER cut-point not reported; [g]ER cut-point ≥1%; [h]ER cut-point ≥10 pmol/mg protein; TNBC, triple negative breast cancer (ER-PR-HER2-); studies in **bold** were significant by multivariate analysis.

Table 2. Univariate and multivariate analyses for relapse-free survival (RFS) and overall survival (OS) (training cohort)

Univariate Cox Regression						
Variable	RFS			OS		
	HR	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Age[a] (n=219)	1.01	0.98-1.04	0.460	1.01	1.00-1.02	0.159
Tumor size[b] (n=205)	2.18	1.27-3.74	**0.005**	2.34	1.24-4.40	**0.009**
Grade[b] (n=198)	4.90	1.19-20.17	**0.028**	3.68	0.89-15.29	0.072
Lymph status[d] (n=208)	3.29	1.82-5.94	**<0.0001**	2.42	1.25-4.68	**0.008**
ERα status[e] (n=204)	0.37	0.21-0.66	**<0.0001**	0.23	0.12-0.43	**<0.0001**
PR status[f] (n=205)	0.36	0.21-0.61	**<0.0001**	0.22	0.12-0.42	**<0.0001**
HER-2/neu status[g] (n=201)	2.75	1.56-4.84	**<0.0001**	3.06	1.60-5.87	**0.001**
Ki67 status[h] (n=190)	2.73	1.57-4.73	**<0.0001**	3.42	1.77-6.61	**<0.0001**
AR status[i] (n=210)	0.992	0.98-1.00	**0.015**	0.99	0.98-1.00	**0.001**
AR status 1[j] (210)	0.48	0.25-0.92	**0.026**	0.36	0.18-0.73	**0.004**
AR status 10[k] (n=210)	0.585	0.32-1.06	0.077	0.39	0.20-0.73	**0.004**
AR status 78[l] (n=210)	0.54	0.32-0.92	**0.024**	0.32	0.16-0.62	**0.001**
Multivariate Analysis – AR 1%						
Variable	RFS (n=196)			OS (n=174)		
	HR	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Tumor size[b]	1.58	0.85-2.82	0.150	1.43	0.70-2.92	0.313
Tumor grade[c]	1.35	0.31-5.91	0.688	-	-	-
Lymph node status[d]	2.57	1.35-4.91	**0.004**	1.98	1.00-3.91	0.051
ERα status[e]	0.83	0.29-2.37	0.724	0.47	0.16-1.44	0.187
PR status[f]	0.61	0.22-1.73	0.355	0.57	0.18-1.82	0.527
HER-2/neu status[g]	1.77	0.89-3.51	0.108	1.83	0.84-3.96	0.127
Ki67 status[h]	1.89	0.92-3.86	0.082	1.73	0.78-3.83	0.178
AR status 1[l]	0.62	0.36-1.20	0.129	1.002	0.40-2.49	0.997
Multivariate Analysis – AR 10%						
Variable	RFS (n=196)			OS (n=174)		
	HR	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Tumor size[b]	1.57	0.85-2.92	0.152	1.43	0.72-2.88	0.309
Tumor grade[c]	1.36	0.31-5.93	0.684	-	-	-
Lymph node status[d]	2.60	1.37-4.94	**0.004**	1.97	0.99-3.91	0.053
ERα status[e]	0.78	0.28-2.17	0.638	0.48	0.16-1.46	0.196
PR status[f]	0.60	0.21-1.69	0.332	0.58	0.18-1.86	0.358
HER-2/neu status[g]	1.74	0.86-3.50	0.122	1.85	0.86-3.95	0.114
Ki67 status[h]	1.88	0.92-3.83	0.085	1.73	0.78-3.83	0.177
AR status 10[l]	0.62	0.36-1.20	0.129	0.95	0.40-12.22	0.898
Multivariate Analysis –AR 78%						
Variable	RFS (n=196)			OS (n=174)		
	HR	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Tumor size[b]	1.53	0.80-2.76	0.181	1.38	0.74-2.57	0.369
Tumor grade[c]	1.25	0.29-5.51	0.788	-	-	-
Lymph node status[d]	2.68	1.41-5.08	**0.003**	1.89	0.95-3.73	0.068
ERα status[e]	0.88	0.33-2.50	0.797	0.52	0.18-1.52	0.228
PR status[f]	0.64	0.22-1.73	0.402	0.69	0.22-2.17	0.527
HER-2/neu status[g]	1.80	0.90-3.56	0.085	2.11	1.01-4.38	**0.046**
Ki67 status[h]	1.95	0.96-3.90	0.067	1.77	0.81-3.87	0.151
AR status 78[l]	0.62	0.36-1.20	0.129	0.41	0.20-0.84	**0.015**

a= Age at diagnosis (continuous variable); b = tumor size (mm) as a dichotomous variable cut-point <=20 vs >20; c = tumor grade (well or moderate vs poor); d = lymph node status (negative vs positive); e = ERα status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells; f = PR status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells; g = HER-2/*neu* status (negative vs positive); h = Ki67 status (% positive cells) as a dichotomous variable cut-point <7.5 vs ≥7.5% positive cells; i= AR status (% positive cells) as a continuous variable; j = AR status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells; j = AR status (% positive cells) as a dichotomous variable cut-point <10 vs ≥10% positive cells; l = AR status (% positive cells) as a dichotomous variable cut-point <78 vs ≥78% positive cells

Table 3. Univariate and multivariate analyses for OS (validation cohort)

Univariate Cox Regression			
Variable	**HR**	**95% CI**	**P value**
Age[a]	1.01	1.00-1.02	0.168
Tumor size[b] (n=417**)**	1.97	1.37-2.83	**<0.0001**
Grade[c] (n=402)	0.89	0.60-1.34	0.585
Lymph status[d] (n=370)	2.46	1.95-3.92	**<0.0001**
ERα status[e] (n=364)	0.53	0.36-0.77	**<0.0001**
PR status[f] (n=267)	0.63	0.42-0.93	**0.024**
HER-2/neu status[g] (n=343)	2.05	1.25-3.35	**0.004**
Ki67 status[h] (n=367)	1.64	1.15-2.35	**0.007**
AR status[i] (n=376)	0.99	0.99-1.00	**0.008**
AR status 1[j] (376)	0.60	0.33-1.10	0.598
AR status 10[k] (n=376)	0.79	0.50-1.23	0.298
AR status 78[l] (n=376)	0.51	0.34-0.75	**0.001**
Multivariate Analysis (n=222)			
Variable	**HR**	**95% CI**	**P value**
Tumor size[b]	1.90	1.07-3.36	**0.028**
Lymph node status[d]	2.34	1.38-3.98	**0.002**
ERα status[e]	1.35	0.63-2.89	0.438
PR status[f]	0.59	0.30-1.14	0.116
HER-2/neu status[g]	2.72	1.34-5.48	**0.005**
Ki67 status[h]	1.66	0.95-2.91	0.075
AR status 78[l]	0.50	0.29-0.87	**0.014**

a= Age at diagnosis (continuous variable)
a = tumor size (mm) as a dichotomous variable cut-point <=20 vs >20
b = tumor grade (well or moderate vs poor)
c = lymph node status (negative vs positive)
d = ERα status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells
e = PR status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells
f = HER-2/*neu* status (negative vs positive)
g = Ki67 status (% positive cells) as a dichotomous variable cut-point <16 vs ≥16% positive cells
h = AR status (% positive cells) as a continuous variable
i = AR status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells
j = AR status (% positive cells) as a dichotomous variable cut-point <10 vs ≥10% positive cells
k = AR status (% positive cells) as a dichotomous variable cut-point <78 vs ≥78% positive cells

Figure Legends

Figure 1. High AR expression is associated with an increased overall survival (OS) in the training cohort. (A) Receiver operating characteristic (ROC) analysis in the training cohort identified an area under the curve of 0.678 (95% CI; 0.587-0.770, *P*<0.0001). (B) Frequency distribution for AR as assessed by visual scoring in a cohort of 219 breast cancers. The mean, median and range of AR percent positivity by immunostaining are shown. (C) Left panel: an example of weak nuclear AR immunostaining in approximately 20% of the tumor cells. Right panel: an example of breast cancer with uniform nuclear AR immunoreactivity of moderate intensity in virtually 100% of tumor cells. Arrows denote examples of AR positive tumor cells. (D) Kaplan-Meier analysis showing that high AR (≥78% nuclear positivity) was significantly associated with increased OS (log rank statistic=12.60, *P* <0.0001). (E) Kaplan-Meier analysis showing that breast tumors with high AR that were ERα positive (AR≥78% ERα+) are associated with a significantly increased overall patient survival (log rank statistic=32.34, *P* <0.0001) when compared to the remaining tumor groups (AR<78% ERα+; AR≥78% ERα- and AR<78% ERα-). (F) Cox regression analysis for OS comparing relative risk among AR and ERα sub-groups in (E); n=204. ERα+ denotes ERα ≥1% positive tumor nuclei; ERα- denotes ERα negative tumors. AR≥78 denotes AR positivity greater than or equal to 78% and AR<78 refers to AR positivity less than 78%. HR, hazard ratio; CI, confidence interval.

Figure 2. AR immunostaining and overall survival (OS) in the validation cohort. (A) Receiver operating characteristic (ROC) analysis in the validation cohort identified an area under the curve of 0.588 (95% CI; 0.525-0.651, *P*=0.008). (B) Frequency distribution for AR was assessed by visual scoring in the validation cohort of 418 breast tumors. The mean, median and range of percent positivity by AR immunostaining are shown. (C) Left panel: an example of weak nuclear AR immunostaining in approximately 20% of tumor cells. Right panel: an example of strong AR nuclear immunoreactivity in approximately 80% of tumor cells. Arrows denote examples of AR positive tumor cells. (D) Kaplan-Meier analysis showing that high AR was significantly associated with increased OS (log rank statistic=12.07, *P*=0.001). (E) Kaplan-Meier analysis showing that breast tumors with high AR (≥78% nuclear positivity) that were ERα positive (AR≥78% ERα+) are associated with significantly increased OS (log rank statistic=20.12, *P*<0.0001) when compared to the remaining tumor groups (AR<78% ERα+; AR≥78% ERα-

and AR<78% ERα). (F) Cox regression analysis for OS, comparing relative risk among the AR and ERα sub-groups in (E); n=344. ERα+ denotes ERα ≥1% tumor nuclei; ERα– denotes ERα negative tumors. AR≥78 denotes AR positivity greater than or equal to 78% and AR<78 refers to AR positivity less than 78%. HR, hazard ratio; CI, confidence interval.

Figure 3. AR:ERα ratio predicts overall survival (OS). Analysis of the AR:ERα ratio in the combined training and validation cohorts (n=552) with cases separated into tertiles; AR:ERα ratio approximating 1 (i.e. 0.87-1.05), AR:ERα ratio >1.05, AR:ERα ratio <0.87. For the 120 patients with ERα negative tumors, patients were divided into groups according to whether the tumors were AR high (≥ 78% nuclear positivity) or not (< 78% AR nuclear positivity). Upper panel: Kaplan-Meier analysis showing the association between the different subgroups and OS (log rank statistic=37.4, P<0.0001). Lower panel: Cox regression analysis showing the relative risk of death in the different subgroups relative those with an AR:ERα ratio approximating 1. HR, hazard ratio; CI, confidence interval.

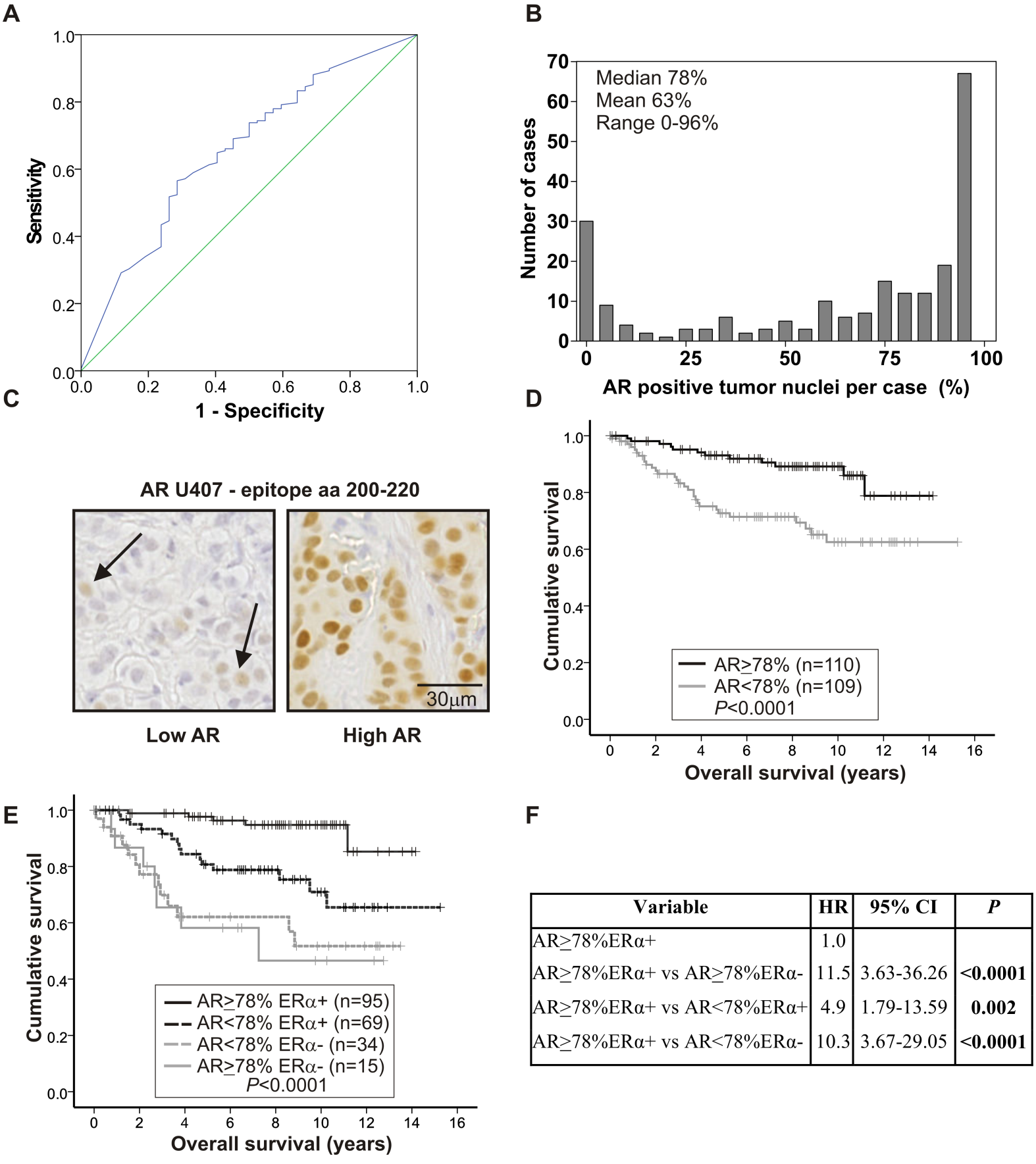
References

1. Collins LC, Cole KS, Marotti JD, Hu R, Schnitt SJ, Tamimi RM. Androgen receptor expression in breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. Mod Pathol 2011; 24:924-31.
2. Honma N, Horii R, Iwase T, Saji S, Younes M, Ito Y, et al. Clinical importance of androgen receptor in breast cancer patients treated with adjuvant tamoxifen monotherapy. Breast Cancer 2013; 20:323-30.
3. Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, et al. Androgen receptor expression and breast cancer survival in postmenopausal women. Clin Cancer Res 2011; 17:1867-74.
4. Moinfar F, Okcu, M., Tsybrovskyy, O., Regitnig, P., Lax, S. F., Weybora, W., Ratschek, M.,, Tavassoli FAaD, H. Androgen receptors frequently are expressed in breast carcinomas: potential relevance to new therapeutic strategies. Cancer 2003; 98:703-11.
5. Park S, Koo J, Park HS, Kim JH, Choi SY, Lee JH, et al. Expression of androgen receptors in primary breast cancer. Ann Oncol 2010; 21:488-92.
6. Park S, Koo JS, Kim MS, Park HS, Lee JS, Kim SI, et al. Androgen receptor expression is significantly associated with better outcomes in estrogen receptor-positive breast cancers. Ann Oncol 2011; 22:1755-62.
7. Tokunaga E, Hisamatsu Y, Taketani K, Yamashita N, Akiyoshi S, Okada S, et al. Differential impact of the expression of the androgen receptor by age in estrogen receptor-positive breast cancer. Cancer medicine 2013; 2:763-73.
8. Hickey TE, Robinson JL, Carroll JS, Tilley WD. Minireview: The androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? Mol Endocrinol 2012; 26:1252-67.
9. McNamara KM, Moore NL, Hickey TE, Sasano H, Tilley WD. Complexities of androgen receptor signalling in breast cancer. Endocrine-related cancer 2014; 21:T161-81.
10. Dimitrakakis C, Bondy C. Androgens and the breast. Breast Cancer Res 2009; 11:212.
11. Tormey DC, Lippman ME, Edwards BK, Cassidy JG. Evaluation of tamoxifen doses with and without fluoxymesterone in advanced breast cancer. Ann Intern Med 1983; 98:139-44.
12. Ingle JN, Twito DI, Schaid DJ, Cullinan SA, Krook JE, Mailliard JA, et al. Combination hormonal therapy with tamoxifen plus fluoxymesterone versus tamoxifen alone in postmenopausal women with metastatic breast cancer. An updated analysis. Cancer 1991; 67:886-91.
13. Overmoyer B, Sanz-Altamira P, Taylor RP, Hancock ML, Dalton JT, Johnston MA, et al. Enobosarm: A targeted therapy for metastatic, androgen receptor positive, breast cancer. 2014 ASCO Annual Meeting; 2014: Journal of Clinical Oncology; 2014. p. abstr 568.
14. Yu Z, He S, Wang D, Patel HK, Miller CP, Brown JL, et al. Selective Androgen Receptor Modulator RAD140 Inhibits the Growth of Androgen/Estrogen Receptor-Positive Breast Cancer Models with a Distinct Mechanism of Action. Clin Cancer Res 2017; 23:7608-20.
15. Cochrane DR, Bernales S, Jacobsen BM, Cittelly DM, Howe EN, NC DA, et al. Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. Breast Cancer Res 2014; 16:R7.
16. Elias AD, Cochrane DR, Jacobsen BM, Cittelly DM, Howe EN, Jean A, et al. Effect of MDV3100, an androgen receptor signaling inhibitor, on tumor growth of estrogen and androgen receptor-positive (ER+/AR+) breast cancer xenografts. J Clin Oncol 2012; 30:abstr 564.
17. D'Amato NC, Gordon MA, Babbs B, Spoelstra NS, Carson Butterfield KT, Torkko KC, et al. Cooperative Dynamics of AR and ER Activity in Breast Cancer. Mol Cancer Res 2016; 14:1054-67.
18. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011; 121:2750-67.
19. Robinson JL, Macarthur S, Ross-Innes CS, Tilley WD, Neal DE, Mills IG, et al. Androgen receptor driven transcription in molecular apocrine breast cancer is mediated by FoxA1. EMBO J 2011; 30:3019-27.
20. Hall RE, Birrell SN, Tilley WD, Sutherland RL. MDA-MB-453, an androgen-responsive human breast carcinoma cell line with high level androgen receptor expression. Eur J Cancer 1994; 30A:484-90.
21. Doane AS, Danso M, Lal P, Donaton M, Zhang L, Hudis C, et al. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. Oncogene 2006; 25:3994-4008.
22. Ni M, Chen Y, Lim E, Wimberly H, Bailey ST, Imai Y, et al. Targeting androgen receptor in estrogen receptor-negative breast cancer. Cancer Cell 2011; 20:119-31.
23. Barton VN, Christenson JL, Gordon MA, Greene LI, Rogers TJ, Butterfield K, et al. Androgen Receptor Supports an Anchorage-Independent, Cancer Stem Cell-like Population in Triple-Negative Breast Cancer. Cancer Res 2017; 77:3455-66.
24. Barton VN, D'Amato NC, Gordon MA, Lind HT, Spoelstra NS, Babbs BL, et al. Multiple molecular subtypes of triple-negative breast cancer critically rely on androgen receptor and respond to enzalutamide in vivo. Mol Cancer Ther 2015; 14:769-78.
25. Thakkar A, Wang B, Picon-Ruiz M, Buchwald P, Ince TA. Vitamin D and androgen receptor-targeted therapy for triple-negative breast cancer. Breast Cancer Res Treat 2016; 157:77-90.
26. Bozovic-Spasojevic I, Zardavas D, Brohee S, Ameye L, Fumagalli D, Ades F, et al. The Prognostic Role of Androgen Receptor in Patients with Early-Stage Breast Cancer: A Meta-analysis of Clinical and Gene Expression Data. Clin Cancer Res 2017; 23:2702-12.
27. Kim Y, Jae E, Yoon M. Influence of Androgen Receptor Expression on the Survival Outcomes in Breast Cancer: A Meta-Analysis. J Breast Cancer 2015; 18:134-42.
28. Qu Q, Mao Y, Fei XC, Shen KW. The impact of androgen receptor expression on breast cancer survival: a retrospective study and meta-analysis. PLoS One 2013; 8:e82650.

29. Vera-Badillo FE, Templeton AJ, de Gouveia P, Diaz-Padilla I, Bedard PL, Al-Mubarak M, et al. Androgen receptor expression and outcomes in early breast cancer: a systematic review and meta-analysis. J Natl Cancer Inst 2014; 106:djt319.
30. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010; 28:2784-95.
31. Iwamoto T, Booser D, Valero V, Murray JL, Koenig K, Esteva FJ, et al. Estrogen receptor (ER) mRNA and ER-related gene expression in breast cancers that are 1% to 10% ER-positive by immunohistochemistry. J Clin Oncol 2012; 30:729-34.
32. Murphy NC, Biankin AV, Millar EK, McNeil CM, O'Toole SA, Segara D, et al. Loss of STARD10 expression identifies a group of poor prognosis breast cancers independent of HER2/Neu and triple negative status. Int J Cancer 2010; 126:1445-53.
33. Makretsov NA, Huntsman DG, Nielsen TO, Yorida E, Peacock M, Cheang MC, et al. Hierarchical clustering analysis of tissue microarray immunostaining data identifies prognostically significant groups of breast carcinoma. Clin Cancer Res 2004; 10:6143-51.
34. Birrell SN, Butler LM, Harris JM, Buchanan G, Tilley WD. Disruption of androgen receptor signaling by synthetic progestins may increase risk of developing breast cancer. FASEB J 2007; 21:2285-93.
35. Elebro K, Borgquist S, Simonsson M, Markkula A, Jirstrom K, Ingvar C, et al. Combined androgen and estrogen receptor status in breast cancer: Treatment prediction and prognosis in a population-based prospective cohort. Clin Cancer Res 2015; 21:3640-50.
36. Husmann DA, Wilson CM, McPhaul MJ, Tilley WD, Wilson JD. Antipeptide antibodies to two distinct regions of the androgen receptor localize the receptor protein to the nuclei of target cells in the rat and human prostate. Endocrinology 1990; 126:2359-68.
37. Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, et al. Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. Cancer Res 2009; 69:6131-40.
38. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009; 101:736-50.
39. Millar EK, Graham PH, McNeil CM, Browne L, O'Toole SA, Boulghourjian A, et al. Prediction of outcome of early ER+ breast cancer is improved using a biomarker panel, which includes Ki-67 and p53. Br J Cancer 2011; 105:272-80.
40. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. Biometrical journal Biometrische Zeitschrift 2005; 47:458-72.
41. Strobl C, Malley J, Tutz G. An introduction to recursive partitioning: rationale, application, and characteristics of classification and regression trees, bagging, and random forests. Psychological methods 2009; 14:323-48.
42. Krogh A, Vedelsby J. Neural network ensembles, cross validation, and active learning. Advances in neural information processing systems 1995; 7:231-8.
43. Bakhtiarizadeh MR, Moradi-Shahrbabak M, Ebrahimi M, Ebrahimie E. Neural network and SVM classifiers accurately predict lipid binding proteins, irrespective of sequence homology. J Theor Biol 2014; 356:213-22.
44. Jamali AA, Ferdousi R, Razzaghi S, Li J, Safdari R, Ebrahimie E. DrugMiner: comparative analysis of machine learning algorithms for prediction of potential druggable proteins. Drug Discov Today 2016; 21:718-24.
45. Asano Y, Kashiwagi S, Onoda N, Kurata K, Morisaki T, Noda S, et al. Clinical verification of sensitivity to preoperative chemotherapy in cases of androgen receptor-expressing positive breast cancer. Br J Cancer 2016; 114:14-20.
46. He J, Peng R, Yuan Z, Wang S, Peng J, Lin G, et al. Prognostic value of androgen receptor expression in operable triple-negative breast cancer: a retrospective analysis based on a tissue microarray. Med Oncol 2012; 29:406-10.
47. Luo X, Shi YX, Li ZM, Jiang WQ. Expression and clinical significance of androgen receptor in triple negative breast cancer. Chin J Cancer 2010; 29:585-90.
48. Tang D, Xu S, Zhang Q, Zhao W. The expression and clinical significance of the androgen receptor and E-cadherin in triple-negative breast cancer. Med Oncol 2012; 29:526-33.
49. Ricciardi GR, Adamo B, Ieni A, Licata L, Cardia R, Ferraro G, et al. Androgen receptor (AR), E-cadherin, and Ki-67 as emerging targets and novel prognostic markers in triple-negative breast cancer (TNBC) patients. PLoS One 2015; 10:e0128368.
50. Choi JE, Kang SH, Lee SJ, Bae YK. Androgen receptor expression predicts decreased survival in early stage triple-negative breast cancer. Annals of surgical oncology 2015; 22:82-9.
51. Jiang HS, Kuang XY, Sun WL, Xu Y, Zheng YZ, Liu YR, et al. Androgen receptor expression predicts different clinical outcomes for breast cancer patients stratified by hormone receptor status. Oncotarget 2016.
52. Collett K, Maehle BO, Skjaerven R, Aas T. Prognostic role of oestrogen, progesterone and androgen receptor in relation to patient age in patients with breast cancer. Breast 1996; 5:123-6.
53. Gonzalez-Angulo AM, Stemke-Hale K, Palla SL, Carey M, Agarwal R, Meric-Berstam F, et al. Androgen receptor levels and association with PIK3CA mutations and prognosis in breast cancer. Clin Cancer Res 2009; 15:2472-8.
54. Soreide JA, Lea OA, Varhaug JE, Skarstein A, Kvinnsland S. Androgen receptors in operable breast cancer: relation to other steroid hormone receptors, correlations to prognostic factors and predictive value for effect of adjuvant tamoxifen treatment. Eur J Surg Oncol 1992; 18:112-8.
55. Aleskandarany MA, Abduljabbar R, Ashankyty I, Elmouna A, Jerjees D, Ali S, et al. Prognostic significance of androgen receptor expression in invasive breast cancer: transcriptomic and protein expression analysis. Breast Cancer Res Treat 2016; 159:215-27.
56. Santagata S, Thakkar A, Ergonul A, Wang B, Woo T, Hu R, et al. Taxonomy of breast cancer based on normal cell phenotype predicts outcome. J Clin Invest 2014; 124:859-70.
57. Farmer P, Bonnefoi H, Becette V, Tubiana-Hulin M, Fumoleau P, Larsimont D, et al. Identification of molecular apocrine breast tumours by microarray analysis. Oncogene 2005; 24:4660-71.

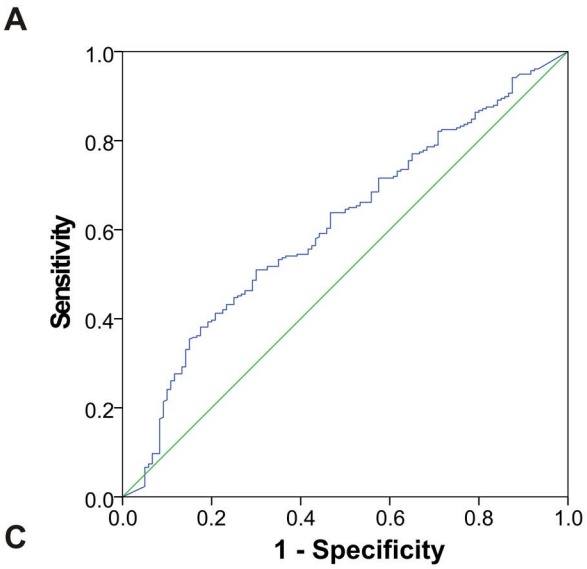
58. Gucalp A, Tolaney S, Isakoff SJ, Ingle JN, Liu MC, Carey LA, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic Breast Cancer. Clin Cancer Res 2013; 19:5505-12.
59. Traina TA, Miller K, Yardley DA, Eakle J, Schwartzberg LS, O'Shaughnessy J, et al. Enzalutamide for the Treatment of Androgen Receptor-Expressing Triple-Negative Breast Cancer. J Clin Oncol 2018:JCO2016713495.
60. Hackenberg R, Luttchens S, Hofmann J, Kunzmann R, Holzel F, Schulz KD. Androgen sensitivity of the new human breast cancer cell line MFM-223. Cancer Res 1991; 51:5722-7.
61. De Amicis F, Thirugnansampanthan J, Cui Y, Selever J, Beyer A, Parra I, et al. Androgen receptor overexpression induces tamoxifen resistance in human breast cancer cells. Breast Cancer Res Treat 2010; 121:1-11.
62. Rechoum Y, Rovito D, Iacopetta D, Barone I, Ando S, Weigel NL, et al. AR collaborates with ERalpha in aromatase inhibitor-resistant breast cancer. Breast Cancer Res Treat 2014; 147:473-85.
63. Fujii R, Hanamura T, Suzuki T, Gohno T, Shibahara Y, Niwa T, et al. Increased androgen receptor activity and cell proliferation in aromatase inhibitor-resistant breast carcinoma. J Steroid Biochem Mol Biol 2014; 144 Pt B:513-22.
64. Allegra JC, Lippman ME, Simon R, Thompson EB, Barlock A, Green L, et al. Association between steroid hormone receptor status and disease-free interval in breast cancer. Cancer Treat Rep 1979; 63:1271-7.
65. Bryan RM, Mercer RJ, Bennett RC, Rennie GC, Lie TH, Morgan FJ. Androgen receptors in breast cancer. Cancer 1984; 54:2436-40.
66. Bryan RM, Mercer RJ, Bennett RC, Rennie GC. Prognostic factors in breast cancer and the development of a prognostic index. Br J Surg 1986; 73:267-71.
67. Langer M, Kubista E, Schemper M, Spona J. Androgen receptors, serum androgen levels and survival of breast cancer patients. Arch Gynecol Obstet 1990; 247:203-9.
68. Kuenen-Boumeester V, Van der Kwast TH, Claassen CC, Look MP, Liem GS, Klijn JG, et al. The clinical significance of androgen receptors in breast cancer and their relation to histological and cell biological parameters. Eur J Cancer 1996; 32A:1560-5.
69. Schippinger W, Regitnig P, Dandachi N, Wernecke KD, Bauernhofer T, Samonigg H, et al. Evaluation of the prognostic significance of androgen receptor expression in metastatic breast cancer. Virchows Arch 2006; 449:24-30.
70. Suzuki T, Miki Y, Moriya T, Akahira J, Ishida T, Hirakawa H, et al. 5Alpha-reductase type 1 and aromatase in breast carcinoma as regulators of in situ androgen production. Int J Cancer 2007; 120:285-91.
71. Carreno G, Del Casar JM, Corte MD, Gonzalez LO, Bongera M, Merino AM, et al. Local recurrence after mastectomy for breast cancer: analysis of clinicopathological, biological and prognostic characteristics. Breast Cancer Res Treat 2007; 102:61-73.
72. Agrawal AK, Jelen M, Grzebieniak Z, Zukrowski P, Rudnicki J, Nienartowicz E. Androgen receptors as a prognostic and predictive factor in breast cancer. Folia Histochem Cytobiol 2008; 46:269-76.
73. Gonzalez LO, Corte MD, Vazquez J, Junquera S, Sanchez R, Alvarez AC, et al. Androgen receptor expresion in breast cancer: relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. BMC Cancer 2008; 8:149.
74. Soiland H, Korner H, Skaland I, Janssen EA, Gudlaugsson E, Varhaug JE, et al. Prognostic relevance of androgen receptor detection in operable breast cancer. J Surg Oncol 2008; 98:551-8.
75. Loibl S, Muller BM, von Minckwitz G, Schwabe M, Roller M, Darb-Esfahani S, et al. Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat 2011; 130:477-87.
76. Yu Q, Niu Y, Liu N, Zhang JZ, Liu TJ, Zhang RJ, et al. Expression of androgen receptor in breast cancer and its significance as a prognostic factor. Ann Oncol 2011; 22:1288-94.
77. Peters KM, Edwards SL, Nair SS, French JD, Bailey PJ, Salkield K, et al. Androgen receptor expression predicts breast cancer survival: the role of genetic and epigenetic events. BMC Cancer 2012; 12:132.
78. Takeshita T, Omoto Y, Yamamoto-Ibusuki M, Yamamoto Y, Iwase H. Clinical significance of androgen receptor and its phosphorylated form in breast cancer. Endocrine-related cancer 2013; 20:L15-21.
79. Lakis S, Kotoula V, Eleftheraki AG, Batistatou A, Bobos M, Koletsa T, et al. The androgen receptor as a surrogate marker for molecular apocrine breast cancer subtyping. Breast 2014.
80. Lakis S, Kotoula V, Eleftheraki AG, Batistatou A, Bobos M, Koletsa T, et al. The androgen receptor as a surrogate marker for molecular apocrine breast cancer subtyping. Breast 2014; 23:234-43.
81. Sultana A, Idress R, Naqvi ZA, Azam I, Khan S, Siddiqui AA, et al. Expression of the Androgen Receptor, pAkt, and pPTEN in Breast Cancer and Their Potential in Prognostication. Translational oncology 2014; 7:355-62.
82. Abduljabbar R, Al-Kaabi MM, Negm OH, Jerjees D, Muftah AA, Mukherjee A, et al. Prognostic and biological significance of peroxisome proliferator-activated receptor-gamma in luminal breast cancer. Breast Cancer Res Treat 2015; 150:511-22.
83. Agoff SN, Swanson PE, Linden H, Hawes SE, Lawton TJ. Androgen receptor expression in estrogen receptor-negative breast cancer. Immunohistochemical, clinical, and prognostic associations. Am J Clin Pathol 2003; 120:725-31.
84. Micello D, Marando A, Sahnane N, Riva C, Capella C, Sessa F. Androgen receptor is frequently expressed in HER2-positive, ER/PR-negative breast cancers. Virchows Arch 2010; 457:467-76.
85. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer 2007; 109:25-32.
86. Mrklic I, Pogorelic Z, Capkun V, Tomic S. Expression of androgen receptors in triple negative breast carcinomas. Acta histochemica 2013; 115:344-8.

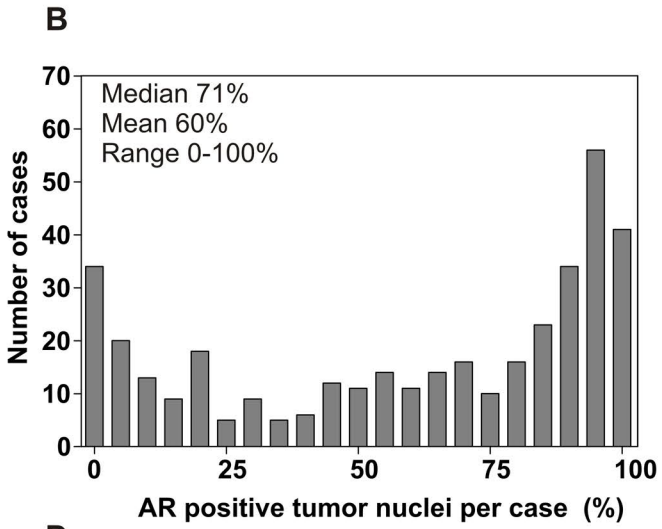
87. McNamara KM, Yoda T, Miki Y, Chanplakorn N, Wongwaisayawan S, Incharoen P, et al. Androgenic pathway in triple negative invasive ductal tumors: its correlation with tumor cell proliferation. Cancer science 2013; 104:639-46.
88. Thike AA, Yong-Zheng Chong L, Cheok PY, Li HH, Wai-Cheong Yip G, Huat Bay B, et al. Loss of androgen receptor expression predicts early recurrence in triple-negative and basal-like breast cancer. Mod Pathol 2014; 27:352-60.
89. Gasparini P, Fassan M, Cascione L, Guler G, Balci S, Irkkan C, et al. Androgen receptor status is a prognostic marker in non-basal triple negative breast cancers and determines novel therapeutic options. PLoS One 2014; 9:e88525.
90. McGhan LJ, McCullough AE, Protheroe CA, Dueck AC, Lee JJ, Nunez-Nateras R, et al. Androgen receptor-positive triple negative breast cancer: a unique breast cancer subtype. Annals of surgical oncology 2014; 21:361-7.
91. Pistelli M, Caramanti M, Biscotti T, Santinelli A, Pagliacci A, De Lisa M, et al. Androgen receptor expression in early triple-negative breast cancer: clinical significance and prognostic associations. Cancers 2014; 6:1351-62.
92. Doberstein K, Milde-Langosch K, Bretz NP, Schirmer U, Harari A, Witzel I, et al. L1CAM is expressed in triple-negative breast cancers and is inversely correlated with androgen receptor. BMC Cancer 2014; 14:958.
93. Zhao L, Niu F, Shen H, Liu X, Chen L, Niu Y. Androgen receptor and metastasis-associated protein-1 are frequently expressed in estrogen receptor negative/HER2 positive breast cancer. Virchows Arch 2016; 468:687-96.
94. Castellano I, Allia E, Accortanzo V, Vandone AM, Chiusa L, Arisio R, et al. Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers. Breast Cancer Res Treat 2010; 124:607-17.
95. Castellano I, Chiusa L, Vandone AM, Beatrice S, Goia M, Donadio M, et al. A simple and reproducible prognostic index in luminal ER-positive breast cancers. Ann Oncol 2013; 24:2292-7.
96. Tsang JY, Ni YB, Chan SK, Shao MM, Law BK, Tan PH, et al. Androgen receptor expression shows distinctive significance in ER positive and negative breast cancers. Annals of surgical oncology 2014; 21:2218-28.
97. Agrawal A, Ziolkowski P, Grzebieniak Z, Jelen M, Bobinski P, Agrawal S. Expression of Androgen Receptor in Estrogen Receptor-positive Breast Cancer. Applied immunohistochemistry & molecular morphology : AIMM / official publication of the Society for Applied Immunohistochemistry 2016; 24:550-5.

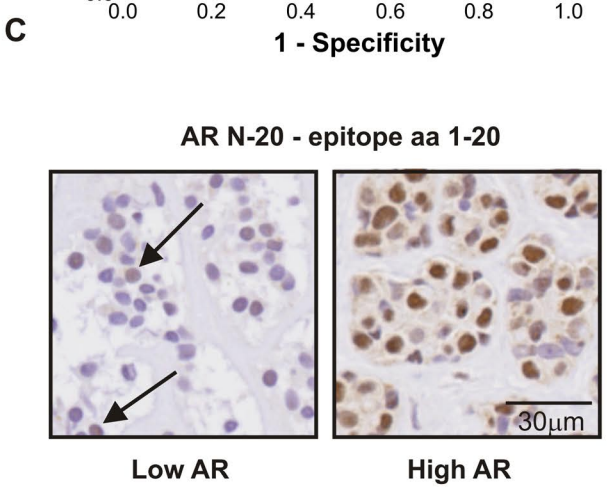


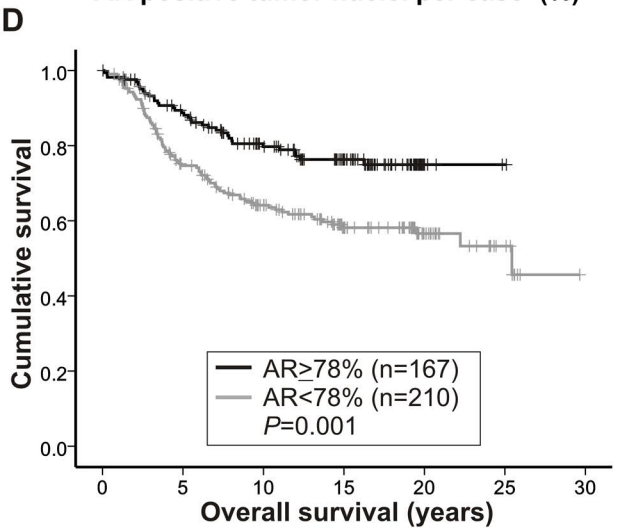
Variable	HR	95% CI	*P*
AR≥78%ERα+	1.0		
AR≥78%ERα+ vs AR≥78%ERα-	11.5	3.63-36.26	**<0.0001**
AR≥78%ERα+ vs AR<78%ERα+	4.9	1.79-13.59	**0.002**
AR≥78%ERα+ vs AR<78%ERα-	10.3	3.67-29.05	**<0.0001**

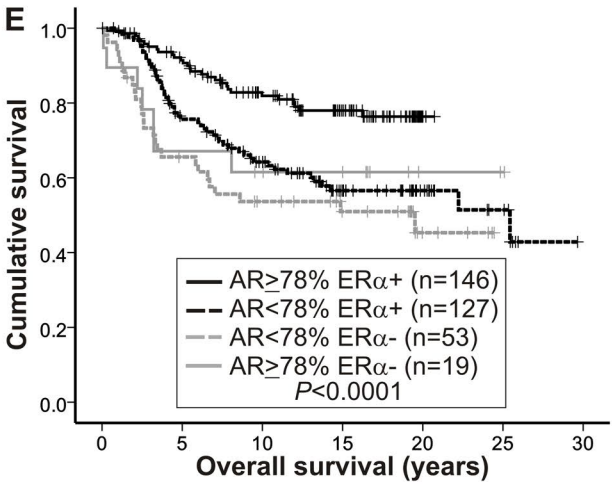
Figure 1







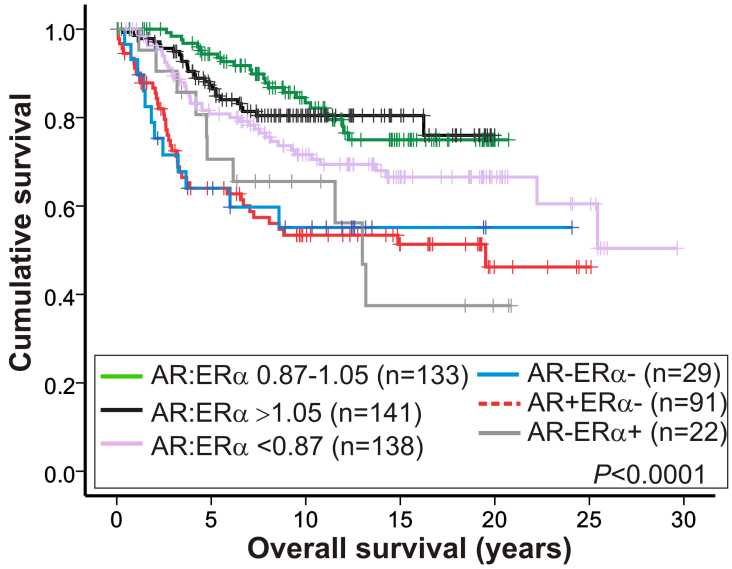




F

Variable	HR	95% CI	*P*
AR≥78% ERα+	1.0		
AR≥78% ERα+ vs AR≥78% ERα-	1.9	1.20-2.95	**0.006**
AR≥78% ERα+ vs AR<78% ERα+	2.4	1.12-4.90	**0.024**
AR≥78% ERα+ vs AR<78% ERα-	2.9	1.67-4.85	**<0.0001**

Figure 2



Variable	Combined training & validation cohorts OS		
	HR	95% CI	*P*
AR:ERα 0.87-1.05	1.00		
AR:ERα 0.87-1.05 vs AR:ERα >1.05	1.12	0.65-1.98	0.68
AR:ERα 0.87-1.05 vs AR:ERα <0.87	1.69	1.02-2.81	**0.042**
AR:ERα 0.87-1.05 vs AR+ERα-	3.24	1.96-5.36	**<0.0001**
AR:ERα 0.87-1.05 vs AR-ERα+	3.00	1.43-6.26	**0.004**
AR:ERα 0.87-1.05 vs AR-ERα-	3.22	1.62-6.48	**0.001**

Figure 3

AACR American Association for Cancer Research

Clinical Cancer Research

The Magnitude of Androgen Receptor Positivity in Breast Cancer is Critical for Reliable Prediction of Disease Outcome

Carmela Ricciardelli, Tina Bianco-Miotto, Shalini Jindal, et al.

Clin Cancer Res Published OnlineFirst March 7, 2018.